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**Pharmaceutical with stabilised granulocytes colony stimulating factor -  
contains surfactant, saccharide, protein or high mol. wt. cpd. as  
stabiliser**

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GB 2193631	A	19880217	GB 8716904	A	19870717	198807	
FR 2601591	A	19880122	FR 8710156	A	19870717	198811	
NL 8701640	A	19880216	NL 871640	A	19870713	198811	
AU 8775665	A	19880121				198812	
NO 8702966	A	19880215				198812	
SE 8702907	A	19880119				198812	
DK 8703683	A	19880119				198815	
ZA 8705268	A	19880127	ZA 875268	A	19870717	198818	
HU 44941	T	19880530				198825	
JP 63146826	A	19880618	JP 87178031	A	19870716	198830	
JP 63146827	A	19880618	JP 87178032	A	19870716	198830	
JP 63146828	A	19880618	JP 87178034	A	19870716	198830	
JP 63152326	A	19880624	JP 87178033	A	19870716	198831	
PT 85343	A	19880729				198835	
BE 1000253	A	19880927	BE 88787	A	19880927	198840	
CN 8704963	A	19880224				198915	
CH 671157	A	19890815				198937	
ES 2010226	A	19891101	ES 872106	A	19870717	199004	
GB 2193631	B	19901121				199047	
IT 1218927	B	19900424				199211	
CA 1297007	C	19920310				199216	
NO 171828	B	19930201	NO 872966	A	19870716	199310	
IL 83220	A	19930131	IL 83220	A	19870717	199311	
KR 9304597	B1	19930601	KR 877804	A	19870718	199423	
RU 2025120	C1	19941230	SU 4203033	A	19870717	199531	
SE 503312	C2	19960513	SE 872907	A	19870717	199625	
AT 8701775	A	19960815	AT 871775	A	19870714	199637	
DK 171308	B	19960902	DK 873683	A	19870715	199641	
JP 2577742	B2	19970205	JP 87178031	A	19870716	199710	
JP 2577743	B2	19970205	JP 87178032	A	19870716	199710	
AT 402259	B	19970215	AT 871775	A	19870714	199713	
JP 2629000	B2	19970709	JP 87178033	A	19870716	199732	
NL 192917	B	19980105	NL 871640	A	19870713	199807	
PH 28830	A	19950314	PH 35555	A	19870717	199844	
DE 3723781	C2	19990902	DE 3723781	A	19870717	199939	

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JP 2577743	B2	6		A61K-038/00	Previous Publ. patent JP 63146827
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JP 2629000	B2	5		A61K-038/00	Previous Publ. patent JP 63152326
NL 192917	B	11		A61K-047/00	
IL 83220	A			A61K-037/02	
KR 9304597	B1			A61K-037/02	
SE 503312	C2			A61K-038/19	
AT 8701775	A			A61K-038/19	
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Abstract (Basic): DE 3723781 A

A pharmaceutical contains stabilised G-CSF (granulocytes colony stimulating factor) as active ingredient and at least one surfactant, saccharide, protein or a high mol. wt. cpd. Pref. the amt. of surfactant or saccharide is 1-10000 pts. wt. Pref. pt. wt. G-CSF. Pref. the surfactant is non-ionic (esp. a sorbitan ester glycerine ester, poly-glycerine ester, polyoxyethylene sorbital ester, polyoxyethylene-glycerine ester or polyethylene glycol ester of an aliphatic acid, polyoxyethylene polyoxypropylene alkyl ether, a hardened polyoxyethylated castor oil, a polyoxyethylated bees wax deriv. a polyoxyethylene lanoline deriv. or an aliphatic polyoxyethylene acid aride), anionic (esp. an alkylsulphate or alkylsulpho succinyl ester salt) or natural (esp. lecithin, sphingophospholipid or an ester of an aliphatic acid with sucrose.

USE/ADVANTAGE - G-CSF can be used to treat various infectious diseases, but is unstable and highly sensitive to changes in the environment e.g. temp., humidity, oxygen or UV light. The invention stabilises the G-CSF and protects it completely against loss of activity.

Title Terms: PHARMACEUTICAL; STABILISED; GRANULOCYTE; COLONY; STIMULATING; FACTOR; CONTAIN; SURFACTANT; SACCHARIDE; PROTEIN; HIGH; MOLECULAR; WEIGHT ; COMPOUND; STABILISED

Derwent Class: A96; B04

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(56) Documents cited

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(58) Field of search

**A5B**

**Selected US specifications from IPC sub-class A61K**

(54) **Stable granulocyte colony stimulating factor composition**

(57) A stable granulocyte colony stimulating factor-containing pharmaceutical preparation contains, in addition to the active agent, at least one substance selected from a pharmaceutically acceptable surfactant, saccharide, protein and high-molecular weight compound.

GB 2 193 631 A

## SPECIFICATION

**Stable pharmaceutical preparation containing granulocyte colony stimulating factor and process for producing the same**

5 The present invention relates to a pharmaceutical preparation containing a granulocyte colony stimulating factor. In particular, the present invention relates to a stabilized pharmaceutical preparation containing a granulocyte colony stimulating factor that is protected against loss or inactivation of the active component (i.e., granulocyte colony stimulating factor) due to adsorption on the wall of a container in which the preparation is put, or to association, polymerization or oxidation of said component. 10

Chemotherapy has been undertaken as one method for treating a variety of infectious diseases but it has recently been found that chemotherapy causes some serious clinical problems such as the generation of drug-resistant organisms, change of causative organisms, and high side effects. 15 In order to avoid these problems associated with chemotherapy involving the use of therapeutic agents such as antibiotics and bactericides, attempts are being made to use a substance that activates the prophylactic capabilities of the host of an infection-causing organism and thereby providing a complete solution to the aforementioned problems of chemotherapy. Of the various prophylactic capabilities of the host, the phagocytic bactericidal action of leucocytes is believed 20 to cause the strongest influence in the initial period of bacterial infection and it is therefore assumed to be important to enhance the infection protecting capabilities of the host by promoting the growth of neutrophils and their differentiation into the mature state. A granulocyte colony stimulating factor (G-CSF) is one of the very useful substances that exhibit such actions and the same assignee of the present invention previously filed a patent application on an 25 infection protecting agent using G-CSF (Japanese Patent Application No. 23777/1985).

As mentioned above, chemotherapy as currently practiced involves various unavoidable problems and intensive efforts are being made to use a drug substance that is capable of activating the prophylactic functions of the host or the person who has been infected.

Needless to say, G-CSF displays by itself the ability to activate the prophylactic functions of the host and it has also been found that G-CSF exhibits greater therapeutic effects in clinical applications if it is used in combination with a substance that activates the prophylactic capabilities of the host. 30

G-CSF is used in a very small amount and a pharmaceutical preparation containing 0.1—500  $\mu$ g (preferably 5—50  $\mu$ g) of G-CSF is usually administered at a dose rate of 1—7 times a week per adult. However, G-CSF has a tendency to be adsorbed on the wall of its container such as an ampule for injection or a syringe. Therefore, if the drug is used as an injection in such a form as an aqueous solution, it will be adsorbed on the wall of its container such as an ampule or a syringe. This either results in the failure of G-CSF to fully exhibit its activity as a pharmaceutical agent or necessitates the incorporation of G-CSF in a more-than-necessary amount making 40 allowance for its possible loss due to adsorption.

In addition, G-CSF is labile and highly susceptible to environmental factors such as temperature, humidity, oxygen and ultraviolet rays. By the agency of such factors, G-CSF undergoes physical or chemical changes such as association, polymerization and oxidation and suffers a great loss in activity. These phenomenon make it difficult to ensure complete accomplishment of 45 a therapeutic act by administering a very small amount of G-CSF in a very exact manner.

It is therefore necessary to develop a stable pharmaceutical preparation of G-CSF that is fully protected against a drop in the activity of its effective component. This is the principal object of the present invention which provides a stable pharmaceutical preparation of G-CSF.

The present inventors conducted intensive studies in order to enhance the stability of a G-CSF containing pharmaceutical preparation and found that this object can effectively be attained by 50 addition of a pharmaceutically acceptable surfactant, saccharide, protein or high-molecular weight compound.

Therefore, the stable G-CSF containing pharmaceutical preparation of the present invention is characterized by containing both G-CSF and at least one substance selected from the group of a 55 pharmaceutically acceptable surfactant, saccharide, protein and high-molecular weight compound.

The G-CSF to be contained in the pharmaceutical preparation of the present invention can be obtained by any of the methods such as those described in the specifications of Japanese Patent Application Nos. 153273/1984, 269455/1985, 269456/1985, 270838/1985 and 270839/1985. For example, a human G-CSF can be prepared either by cultivating a cell strain 60 (CNCM Accession Number I-315 or I-483) collected from tumor cells of patients with oral cavity cancer, or by expressing a recombinant DNA (which has been prepared by the agency of a human G-CSF encoding gene) in an appropriate host cell (e.g. *E. coli*, C 127 cell or ovary cells of a Chinese hamster).

Any human G-CSF that has been purified to high degree may be employed as the G-CSF to be 65 contained in the pharmaceutical preparation of the present invention. Preferable human G-CSFs 65

are ones obtained by isolation from the supernatant of the culture of a human G-CSF producing cell, and a polypeptide or glycoprotein having the human G-CSF activity that is obtained by transforming a host with a recombinant vector having incorporated therein a gene coding for a polypeptide having the human G-CSF activity.

5 Two particularly preferable examples of human G-CSF are shown below: 5

(1) human G-CSF having the following physicochemical properties:

i) molecular weight: about  $19,000 \pm 1,000$  as measured by electrophoresis through a sodium dodecylsulfate—polyacrylamide gel;

10 ii) isoelectric point: having at least one of the three isoelectric points,  $pI = 5.5 \pm 0.1$ ,  $pI = 5.8 \pm 0.1$ , and  $pI = 6.1 \pm 0.1$ ; 10

iii) ultraviolet absorption: having a maximum absorption at 280 nm and a minimum absorption at 250 nm;

iv) amino acid sequence of the 21 residues from N terminus:  $H_2N$ -Thr-Pro-Leu-Gly-Pro-Ala-Ser-Ser-Leu-Pro-Gln-Ser-Phe-Leu-Leu-Lys-Cys-Leu-Glu-Gln-Val

15 (2) human G-CSF containing either a polypeptide having the human granulocyte stimulating factor activity which is represented by all or part of the amino acid sequence shown below, or a glycoprotein having both said polypeptide and a sugar chain portion: 15

(Met) <sub>n</sub>	Thr	Pro	Leu	Gly	Pro	Ala	Ser	Ser	Leu	Pro	
20	Gln	Ser	Phe	Leu	Leu	Lys	Cys	Leu	Glu	Gln	Val
	Arg	Lys	Ile	Gln	Gly	Asp	Gly	Ala	Ala	Leu	Gln
25	Glu	Lys	Leu	(Val	Ser	Glu) <sub>m</sub>	Cys	Ala	Thr	Tyr	Lys
	Leu	Cys	His	Pro	Glu	Glu	Leu	Val	Leu	Leu	Gly
30	His	Ser	Leu	Gly	Ile	Pro	Trp	Ala	Pro	Leu	Ser
	Ser	Cys	Pro	Ser	Gln	Ala	Leu	Gln	Leu	Ala	Gly
35	Cys	Leu	Ser	Gln	Leu	His	Ser	Gly	Leu	Phe	Leu
	Tyr	Gln	Gly	Leu	Leu	Gln	Ala	Leu	Glu	Gly	Ile
	Ser	Pro	Glu	Leu	Gly	Pro	Thr	Leu	Asp	Thr	Leu
40	Gln	Leu	Asp	Val	Ala	Asp	Phe	Ala	Thr	Thr	Ile
	Trp	Gln	Gln	Met	Glu	Glu	Leu	Gly	Met	Ala	Pro
45	Ala	Leu	Gln	Pro	Thr	Gln	Gly	Ala	Met	Pro	Ala
	Phe	Ala	Ser	Ala	Phe	Gln	Arg	Arg	Ala	Gly	Gly
50	Val	Leu	Val	Ala	Ser	His	Leu	Gln	Ser	Phe	Leu
	Glu	Val	Ser	Tyr	Arg	Val	Leu	Arg	His	Leu	Ala
55	Gln	Pro									

(provided that m is 0 or 1; and n is 0 or 1).

For details of the method for preparing these two types of G-CSF, see the specification of Japanese Patent Application Nos. 153273/1984, 269455/1985, 269456/1985, 270838/1985 60 and 270839/1985, all having been filed by the assignee of the present invention.

Another method that can be employed consists of performing fusion of a G-CSF producing cell with a self-proliferating malignant tumor cell and cultivating the resulting hybridoma in the presence or absence of mytogen.

The human G-CSF containing solution obtained may be stored in a frozen state after being 65 further purified and concentrated, as required, by any known technique. Alternatively, the solu-

tion may be stored after being dehydrated by such means as freeze-drying.

All of the human G-CSFs thus prepared can be processed as specified by the present invention in order to attain stable G-CSF containing pharmaceutical preparations.

- Typical examples of the surfactant that is used to attain the stable G-CSF containing pharmaceutical preparation of the present invention are listed below: nonionic surfactants with HLB of 5 6—18 such as sorbitan aliphatic acid esters (e.g. sorbitan monocaprylate, sorbitan monolaurate and sorbitan monopalmitate), glycerin aliphatic acid esters (e.g. glycerin monocaprylate, glycerin monomyristate, and glycerin monostearate), polyglycerin aliphatic acid esters (e.g. decaglyceryl monostearate, decaglyceryl distearate and decaglyceryl monolinoleate), polyoxyethylene sorbitan 10 aliphatic acid esters (e.g. polyoxyethylene sorbitan monolaurate, polyoxyethylene sorbitan monooleate, polyoxyethylene sorbitan monostearate, polyoxyethylene sorbitan monopalmitate, polyoxyethylene sorbitan trioleate, and polyoxyethylene sorbitan tristearate), polyoxyethylene sorbitol aliphatic acid esters (e.g. polyoxyethylene sorbitol tetrastearate and polyoxyethylene sorbitol tetraoleate), polyethylene glycerin aliphatic acid esters (e.g. polyoxyethylene glyceryl monostearate), 15 polyethylene glycol aliphatic acid esters (e.g. polyethylene glycol distearate), polyoxyethylene alkyl ethers (e.g. polyoxyethylene lauryl ether), polyoxyethylene polyoxypropylene alkyl ethers (e.g. polyoxyethylene polyoxypropylene glycol ether, polyoxyethylene polyoxypropylene propyl ether, and polyoxyethylene polyoxypropylene cetyl ether), polyoxyethylene alkylphenyl ethers (e.g. polyoxyethylene nonylphenyl ether), polyoxyethylated castor oil, polyoxyethylated hardened castor 20 oil (polyoxyethylated hydrogenated castor oil), polyoxyethylated beeswax derivatives (e.g. polyoxyethylated sorbitol beeswax), polyoxyethylene lanolin derivatives (e.g. polyoxyethylene lanolin), and polyoxyethylene aliphatic acid amides (e.g. polyethylene stearic acid amide); nonionic surfactants such as alkyl sulfuric acid salts having a C<sub>10</sub>—C<sub>18</sub> alkyl group (e.g. sodium cetyl sulfate, sodium lauryl sulfate and sodium oleyl sulfate), polyoxyethylene alkyl ether sulfuric acid salts 25 wherein the average molar number of ethylene oxide addition is 2—4 and the alkyl group has 10—18 carbon atoms (e.g. polyoxyethylene sodium lauryl sulfate), salts of alkyl sulfosuccinate esters wherein the alkyl group has 8 -18 carbon atoms (e.g. sodium lauryl sulfosuccinate ester); and natural surfactants such as lecithin, glycerophospholipid, sphingophospholipid (e.g. sphingomyelin), and sucrose aliphatic acid esters wherein the aliphatic acid has 12—18 carbon atoms. 30 These surfactants may of course be used either independently or in admixture.

The surfactants listed above are preferably used in amounts of 1—10,000 parts by weight per part by weight of G-CSF.

- The saccharide to be used in making the stable G-CSF containing pharmaceutical preparation of the present invention may be selected from among monosaccharides, oligosaccharides, and 35 polysaccharides, as well as phosphate esters and nucleotide derivatives thereof so long as they are pharmaceutically acceptable. Typical examples are listed below: trivalent and higher sugar alcohols such as glycerin, erythritol, arabitol, xylitol, sorbitol, and mannitol; acidic sugars such as glucuronic acid, iduronic acid, neuraminic acid, galacturonic acid, gluconic acid, mannuronic acid, ketoglycolic acid, ketogalactonic acid and ketogulonic acid; hyaluronic acid and salts thereof, 40 chondroitin sulfate and salts thereof, heparin, inulin, chitin and derivatives thereof, chitosan and derivatives thereof, dextrin, dextran with an average molecular weights of 5,000 - 150,000, and alginic acid and salts thereof. All of these saccharides may be used with advantage either independently or in admixture.

- The saccharides listed above are preferably used in amounts of 1—10,000 parts by weight 45 per part by weight of G-CSF.

- Typical examples of the protein to be used in making the stable G-CSF containing pharmaceutical preparation of the present invention include human serum albumin, human serum globulin, gelatin, acid-treated gelatin (average mol. wt. = 7,000—100,000), alkali-treated gelatin (average mol. wt. = 7,000—100,000), and collagen. Needless to say, these proteins may be used either 50 independently or in admixture.

The proteins listed above are preferably used in amounts of 1—20,000 parts by weight per part by weight of G-CSF.

- Typical examples of the high-molecular weight compound to be used in making the stable G-CSF containing pharmaceutical preparation of the present invention include: natural polymers such 55 as hydroxypropyl cellulose, hydroxymethyl cellulose, sodium carboxymethyl cellulose, and hydroxyethyl cellulose; and synthetic polymers such as polyethylene glycol (mol. wt. = 300—6,000), polyvinyl alcohol (mol. wt. = 20,000—100,000), and polyvinylpyrrolidone (mol. wt. = 20,000—100,000). Needless to say, these high-molecular weight compounds may be used either alone or in combination.

- The high-molecular weight compounds listed above are desirably used in amounts of 60 1—20,000 parts by weight per part by weight of G-CSF.

- In addition to the surfactant, saccharide, protein or high-molecular weight compound described above, at least one member selected from the group consisting of an amino acid, a sulfurous reducing agent and an antioxidant may also be incorporated in making the G-CSF containing 65 pharmaceutical preparation of the present invention. Illustrative amino acids include glycine,

threonine, tryptophan, lysine, hydroxylysine, histidine, arginine, cysteine, cystine, and methionine. Illustrative sulfureous reducing agents include: N-acetylcysteine, N-acetylhomocysteine, thiocetic acid, thiodiglycol, thioethanolamine, thioglycerol, thiosorbitol, thioglycolic acid and salts thereof, sodium thiosulfate, sodium hydrogensulfite, sodium pyrosulfite, sodium sulfite, thiolactic acid, dithiothreitol, glutathione, and a mild sulfureous reducing agent having a sulfhydryl group such as a C<sub>1</sub>—C<sub>7</sub> thioalkanoic acid. Illustrative anti-oxidants include erythorbic acid, dibutylhydroxytoluene, butylhydroxyanisole, dl- $\alpha$ -tocopherol, tocopherol acetate, L-ascorbic acid and salts thereof, L-ascorbic acid palmitate, L-ascorbic acid stearate, triamyl gallate, propyl gallate and chelating agents such as disodium ethylenediaminetetraacetate (EDTA), sodium pyrophosphate and sodium metaphosphate.

The above-listed amino acids, sulfureous reducing agents and antioxidants or mixtures thereof are preferably used in amounts of 1—10,000 parts by weight per part by weight of G-CSF.

For the purpose of formulating the stable G-CSF containing preparation of the present invention in a suitable dosage form, one or more the following agents may be incorporated: a diluent, a solubilizing aid, an isotonic agent, an excipient, a pH modifier, a soothing agent, and a buffer.

The stabilized G-CSF pharmaceutical preparation of the present invention may be formulated either for oral administration or for parenteral administration such as by injection applied in various ways, and a variety of dosage forms may be employed depending upon the specific mode of administration. Typical dosage forms include: those intended for oral administration such as tablets, pills, capsules, granules and suspensions; solutions, suspensions and freeze-dried preparations principally intended for intravenous injection, intramuscular injection, subcutaneous injection and intracutaneous injection; and those intended for transmucosal administration such as rectal suppositories, nasal drugs, and vaginal suppositories.

According to the present invention, at least one substance selected from the group consisting of a surfactant, a saccharide, a protein or a high-molecular weight compound is added to a G-CSF containing pharmaceutical preparation so that it is prevented from being adsorbed on the wall of its container or a syringe while at the same time, it remains stable over a prolonged period of time.

The detailed mechanism by which the substances mentioned above stabilized G-CSF or prevent it from being adsorbed is yet to be clarified. In the presence of a surfactant, the surface of G-CSF which is a hydrophobic protein would be covered with the surfactant to become solubilized so that the G-CSF present in a trace amount is effectively prevented from being adsorbed on the wall of its container or a syringe. A saccharide or hydrophilic high-molecular weight compound would form a hydrated layer between G-CSF and the adsorptive surface of the wall of its container or a syringe, thereby preventing adsorption of G-CSF in an effective manner. A protein would compete with G-CSF for adsorption on the wall of its container or a syringe, thereby effectively inhibiting adsorption of G-CSF.

Besides the prevention of G-CSF adsorption, the substances mentioned above would also contribute to the prevention of association or polymerization of the molecules of G-CSF. In the presence of a surfactant, saccharide, protein or high-molecular weight compound, the individual molecules of G-CSF are dispersed in these substances and the interaction between the G-CSF molecules is sufficiently reduced to cause a significant decrease in the probability of their association or polymerization. In addition, these substances would retard the autoxidation of G-CSF that is accelerated under high temperature or humidity or prevent G-CSF from being associated or polymerized as a result of its autoxidation. These effects of retarding autoxidation of G-CSF or preventing it from being associated or polymerized would be further enhanced by addition of an amino acid, a sulfureous reducing agent or an antioxidant.

The problems described above are particularly noticeable in solutions for injection and in suspensions but they also occur during the process of formulating G-CSF in other dosage forms such as tablets. The addition of surfactants, saccharides, proteins or high-molecular weight compounds is also effective in this latter case.

Through the addition of at least one substance selected from the group consisting of a surfactant, saccharide, protein and a high-molecular weight compound, G-CSF is highly stabilized and maintains its activity for a prolonged period of time, as will be demonstrated in the examples that follow. To attain these results, the amount of each of these substances, in particular its lower limit, is critical and the following ranges are desirable: 1—10,000 parts by weight of surfactant, 1—10,000 parts by weight of saccharide, 1—20,000 parts by weight of protein, and 1—20,000 parts by weight of high-molecular weight compound, per 1 part by weight of G-CSF.

According to the present invention, a surfactant, a saccharide, a protein and/or a high-molecular weight compound is used in a specified concentration and this is effective not only in preventing G-CSF from being adsorbed on the wall of its container or a syringe but also in enhancing the stability of a G-CSF containing pharmaceutical preparation. As a result, it becomes possible to ensure the administration of a small but highly precise dose of G-CSF to patients; since G-CSF is costly, its efficient utilization will lead to lower costs for the production of G-CSF

containing pharmaceutical preparations.

The following examples are provided for the purpose of further illustrating the present invention but are in no sense to be taken as limiting. In these examples, the residual activity of G-CSF was determined by one of the following methods.

5 (a) *Soft agar method using mouse bone marrow cells:*

A horse serum (0.4 ml), 0.1 ml of the sample, 0.1 ml of a C3H/He (female) mouse bone marrow cell suspension ( $0.5 - 1 \times 10^5$  nuclear cells), and 0.4 ml of a modified McCoy's 5A culture solution containing 0.75% of agar were mixed, poured into a plastic dish for tissue culture (35 mm<sup>2</sup>), coagulated, and cultured for 5 days at 37°C in 5% CO<sub>2</sub>/95% air and at 100% humidity. The number of colonies formed was counted (one colony consisting of at least 50 cells) and the activity was determined with one unit being the activity for forming one colony.

The modified McCoy's 5A culture solution used in the method (a) was prepared by the following procedures.

15 *Modified McCoy's 5A culture solution (double concentration)*

Twelve grams of McCoy's 5A culture solution (Gibco), 2.55 g of MEM amino acid-vitamin medium (Nissui Seiyaku Co., Ltd.), 2.18 g of sodium bicarbonate and 50,000 units of potassium penicillin G were dissolved twice in 500 ml of distilled water and the solution was aseptically filtered through a Millipore filter (0.22 μm).

20 (b) *Reverse-phase high-performance liquid chromatography:*

Using a reverse-phase C8 column (4.6 mm x 300 mm; 5 μm) and an n-propanol/trifluoroacetic acid mixture as a mobile phase, the residual activity of G-CSF (injected in an amount equivalent to 1 μg) was determined under the following gradient conditions:

25	Time (sec)	Solvent (A)	Solvent (B)	Gradient
	0	100%	0%	} linear
30	15	0%	100%	
	25	100%	0%	

35 Solvent (A): 30% n-propanol and 0.1% trifluoroacetic acid

Solvent (B): 60% n-propanol and 0.1% trifluoroacetic acid

Detection was conducted at a wavelength of 210 nm and the percentage of the residual G-CSF activity was calculated by the following formula:

$$40 \quad \text{Residual G-CSF activity (\%)} = \frac{\text{the residual amount of G-CSF after the lapse of a given time}}{\text{the initial amount of G-CSF}} \times 100$$

45 The residual amount of G-CSF as determined by this method correlated very well with the result attained in measurement by the soft agar method (a) using mouse bone marrow cells.

*Example 1*

To 5 μg of G-CSF, one of the stabilizing agents listed in Table 1 was added and the mixture was aseptically dissolved in a 20 mM buffer solution (containing 100 mM sodium chloride; pH 7.4) to make a pharmaceutical preparation containing 5 μg of G-CSF per ml, which was then freezer-dried. The time-dependent change in G-CSF activity was measured by method (a) and the results are shown in Table 1. The term "activity (%)" in the table represents the residual activity of G-CSF relative to the initial unit and is defined by the following formula:

$$55 \quad \text{Activity (\%)} = \frac{\text{activity unit after the lapse of a given time}}{\text{initial activity unit}} \times 100$$

60 Freeze-drying was conducted by the following procedures:

The G-CSF solution containing a stabilizing agent was put into a sterile sulfa-treated glass vial, frozen at -40°C or below for 4 hours, subjected to primary drying by heating from -40°C to 0°C over a period of 48 hours with the pressure increased from 0.03 to 0.1 torr, then to

65 secondary during by heating from 0°C to 20°C for a period of 12 hours with the pressure

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increased from 0.03 to 0.08 torr; thereafter, the interior of the vial was filled with a sterile dry nitrogen gas to attain an atmospheric pressure and the vial was plugged with a freeze-drying rubber stopper, then sealed with an aluminum cap.

Table 1

5	Stabilizing agent	Amount (parts by weight)	Activity (%)		5
			After storage at 4°C for 6 months	After storage at 37°C for 1 month	
10	xylitol	10,000	92	86	10
	mannitol	10,000	91	85	
15	glucuronic acid	10,000	86	82	15
	hyaluronic acid	2,000	92	89	
20	dextran (m.w. 40,000)	2,000	95	90	20
	heparin	5,000	85	80	
	chitosan	2,000	93	91	
25	alginic acid	2,000	90	90	25
	human serum albumin	1,000	98	99	
30	human serum globulin	1,000	98	95	30
	acid-treated gelatin	2,000	97	95	
35	alkali-treated gelatin	1,000	99	96	35
	collagen	2,000	95	90	
40	polyethylene glycol (m.w. 4,000)	10,000	94	90	40
	hydroxypropyl cellulose	1,000	98	94	
45	sodium carboxymethyl cellulose	1,000	88	80	45
	hydroxymethyl cellulose	5,000	92	90	
50	polyvinyl alcohol (m.w. 50,000)	2,000	96	95	50
55	polyvinylpyrrolidone (m.w. 50,000)	2,000	95	94	55
60	human serum albumin	2,000	100	97	60
	mannitol	2,000			
	cysteine	100			

Table 1 (cont'd)

5	Stabilizing agent	Amount (parts by weight)	Activity (%)		5
			After storage at 4°C for 6 months	After storage at 37°C for 1 month	
10	human serum albumin	2,000			10
15	polyoxyethylene sorbitan monolaurate	100	99	96	15
	mannitol	2,000			
20	human serum albumin	2,000			20
	hydroxypropyl cellulose	500	98	92	
	dextran (m.w. 40,000)	2,000			
25	polyoxyethylene sorbitan monolaurate	100			25
	sorbitol	2,000	98	96	
30	polyoxyethylated hardened castor oil	100			30
	dextran (m.w. 40,000)	2,000	94	92	
35	not added	-	74	58	35

*Example 2*

40 To 10 µg of G-CSF, one of the stabilizing agents listed in Table 2 was added and the mixture was aseptically dissolved in a 20 mM phosphate buffer solution (containing 100 mM sodium chloride; pH, 7.4) to make a pharmaceutical preparation containing 10 µg of G-CSF per ml. The preparation was aseptically charged into a sulfa-treated glass vial and sealed to make a G-CSF solution. The time-dependent change in the activity of G-CSF in this solution was measured by 45 the same method as used in Example 1 and the results are shown in Table 2.

Table 2

Stabilizing agent	Amount (parts by weight)	Activity (%)		
		After storage at 4°C for 7 days	After storage at 4°C for 2 months	After storage at RT for 1 month
mannitol	5,000	91	87	82
hyaluronic acid	2,000	93	87	70
dextran (m.w. 40,000)	2,000	96	95	85
glycerin	10,000	90	90	88
neuraminic acid	5,000	93	91	84
chitin	2,000	95	92	86
dextrin	2,000	90	92	87
human serum albumin	1,000	99	95	92
human serum globulin	1,000	98	94	90
acid-treated gelatin	2,000	97	96	87
alkali-treated gelatin	500	99	95	92
collagen	2,000	99	94	88
polyethylene glycol (m.w. 4,000)	10,000	94	89	90
hydroxypropyl cellulose	2,000	98	95	92
sodium carboxymethyl cellulose	2,000	92	91	80
hydroxyethyl cellulose	4,000	92	94	90
polyvinyl alcohol (m.w. 50,000)	4,000	97	93	90
polyvinylpyrrolidone (m.w. 50,000)	4,000	95	95	92
sorbitan monolaurate	400	97	96	95
polyoxyethylene sorbitan monolaurate	400	100	96	94

Table 2 (cont'd)

5	Stabilizing agent	Amount (parts by weight)	Activity (%)			5
			After storage at 4°C for 7 days	After storage at 4°C for 2 months	After storage at RT for 1 month	
10	polyoxyethylene sorbitan monostearate	400	98	97	94	10
15	polyoxyethylene polyoxypropylene glycol ether	400	100	94	93	15
20	polyoxyethylated hardened castor oil	400	99	98	90	20
	sodium lauryl sulfate	2,000	97	93	87	
25	lecithin	2,000	97	94	90	25
	human serum albumin	2,000				
	mannitol	2,000	100	99	97	
30	cysteine	100				30
	human serum albumin	2,000				
35	polyoxyethylene sorbitan monolaurate	100	99	97	95	35
	mannitol	2,000				
40	human serum albumin	1,000				40
	hydroxypropyl cellulose	500	99	97	95	
	dextran (m.w. 40,000)	2,000				
45	polyoxyethylene sorbitan monopalmitate	100				45
	sorbitol	2,000	96	96	93	
50	polyoxyethylated hardened castor oil	100				50
	dextran (m.w. 40,000)	2,000	95	92	92	
55	not added	-	72	61	47	55

*Example 3*

To 10  $\mu\text{g}$  of G-CSF, one of the stabilizing agents listed in Table 3 was added and the mixture was aseptically dissolved in a 20 mM phosphate buffer solution (containing 100 mM sodium chloride; pH, 7.4) to make a pharmaceutical preparation containing 10  $\mu\text{g}$  of G-CSF per ml. One  
5 milliliter of the preparation was charged into a sulfa-treated silicone-coated glass vial and left at 4°C. The effectiveness of each stabilizing agent in preventing G-CSF adsorption was evaluated by measuring the residual activity of G-CSF in the solution after 0.5, 2 and 24 hours. The measurement was conducted by method (b) using reverse-phase high-performance liquid chromatography. The results are shown in Table 3.

5

Table 3

5	Stabilizing agent	Amount (parts by weight)	Residual activity (%)				5
			initial	0.5 h	2 h	24 h	
	monnitrol	5,000	100	93	90	91	
10	hyaluronic acid	2,000	100	97	92	92	10
	dextran (m.w. 40,000)	2,000	100	98	95	96	
15	glycerin	10,000	100	94	91	90	15
	heparin	2,000	100	92	90	90	
20	glucuronic acid	5,000	100	96	90	91	20
	ketoglycolic acid	5,000	100	92	88	90	
25	human serum albumin	1,000	100	100	101	99	25
	human serum globulin	1,000	100	98	100	98	
30	alkali-treated gelatin	500	100	99	98	99	30
	acid-treated gelatin	2,000	100	99	97	97	
	collagen	2,000	100	100	98	99	
35	polyethylene glycol (m.w. 4,000)	10,000	100	100	100	99	35
	hydroxypropyl cellulose	2,000	100	100	100	99	
40	sodium carboxymethyl cellulose	2,000	100	98	96	95	40
	hydroxyethyl cellulose	4,000	100	96	93	92	
45	polyvinyl alcohol (m.w. 50,000)	4,000	100	99	100	98	45
50	polyvinylpyrrolidone (m.w. 50,000)	4,000	100	98	98	96	50
	sorbitan monocaprylate	400	100	100	100	98	
55	polyoxyethylene sorbitan monostearate	400	100	100	98	100	55
60	polyoxyethylated hardened castor oil	400	100	99	101	99	60

Table 3 (cont'd)

5	Stabilizing agent	Amount (parts by weight)	Residual activity (%)				5
			initial	0.5 h	2 h	24 h	
	sodium lauryl sulfate	2,000	100	100	99	97	
10	lecithin	2,000	100	99	100	98	10
	human serum albumin	2,000					
15	mannitol	2,000	100	100	100	101	15
	cysteine	100					
	human serum albumin	2,000					
20	polyoxyethylene	100	100	100	98	99	20
	sorbitan monolaurate						
	mannitol	2,000					
25	human serum albumin	1,000					25
	hydroxypropyl cellulose	500	100	101	99	100	
30	dextran (m.w. 40,000)	2,000					30
	polyoxyethylene	100					
	sorbitan monolaurate		100	100	99	99	
35	sorbitol	2,000					35
	polyoxyethylated	100					
	hardened castor oil		100	100	98	97	
40	dextran (m.w. 40,000)	2,000					40
	not added	-	100	91	72	73	

## CLAIMS

1. A stable granulocyte colony stimulating factor containing pharmaceutical preparation that contains, in addition to the granulocyte colony stimulating factor present as the effective ingredient, at least one substance selected from the group consisting of a pharmaceutically acceptable surfactant, saccharide, protein and high-molecular weight compound. 5
2. A stable granulocyte colony stimulating factor containing pharmaceutical preparation according to Claim 1 which contains the surfactant in an amount of 1—10,000 parts by weight per part by weight of the granulocyte colony stimulating factor.
3. A stable granulocyte colony stimulating factor containing pharmaceutical preparation according to Claim 1 or 2 wherein said surfactant is at least one member selected from the group consisting of a nonionic surfactant, an anionic surfactant and a natural surfactant, the nonionic surfactant being a sorbitan aliphatic acid ester, a glycerin aliphatic acid ester, a polyglycerin aliphatic acid ester, a polyoxyethylene sorbitan aliphatic acid ester, a polyoxyethylene sorbitol aliphatic acid ester, a polyoxyethylene glycerin aliphatic acid ester, a polyethylene glycol aliphatic acid ester, a polyoxyethylene alkyl ether, a polyoxyethylene polyoxypropylene alkyl ether, a polyoxyethylene alkylphenyl ether, a polyoxyethylated hardened castor oil, a polyoxyethylated beeswax derivative, a polyoxyethylene lanolin derivative, or a polyoxyethylene aliphatic acid amide, the anionic surfactant being an alkyl sulfate salt, a polyoxyethylene alkyl ether sulfate salt, or an alkyl sulfosuccinate ester salt, and the natural surfactant being lecithin, glycerophospholipid, sphingophospholipid, or a sucrose aliphatic acid ester. 10 15 20
4. A stable granulocyte colony stimulating factor containing pharmaceutical preparation according to Claim 1 which contains the saccharide in an amount of 1—10,000 parts by weight per part by weight of the granulocyte colony stimulating factor.
5. A stable granulocyte colony stimulating factor containing pharmaceutical preparation according to Claim 1 or 4 wherein said saccharide is at least one member selected from the group consisting of glycerin, erythritol, arabitol, xylitol, sorbitol, mannitol, glucuronic acid, iduronic acid, galacturonic acid, neuraminic acid, glyconic acid, mannuronic acid, ketoglycolic acid, ketogalactonic acid, ketogulonic acid, hyaluronic acid and salts thereof, chondroitin sulfate and salts thereof, heparin, inulin, chitin and derivatives thereof, chitosan and derivatives thereof, dextrin, dextran with an average molecular weight of 5,000 -150,000, and alginic acid and salts thereof. 25 30
6. A stable granulocyte colony stimulating factor containing pharmaceutical preparation according to Claim 1 which contains the protein in an amount of 1—20,000 parts by weight per part by weight of the granulocyte colony stimulating factor.
7. A stable granulocyte colony stimulating factor containing pharmaceutical preparation according to Claim 1 or 6 wherein said protein is at least one member selected from the group consisting of human serum albumin, human serum globulin, gelatin, acid- or alkali-treated gelatin with an average molecular weight of 7,000—100,000, and collagen. 35
8. A stable granulocyte colony stimulating factor containing pharmaceutical preparation according to Claim 1 which contains the high-molecular weight compound in an amount of 1—20,000 parts by weight per part by weight of the granulocyte colony stimulating factor. 40
9. A stable granulocyte colony stimulating factor containing pharmaceutical preparation according to Claim 1 or 8 wherein said high-molecular weight compound is at least one member selected from the group consisting of hydroxypropyl cellulose, hydroxymethyl cellulose, sodium carboxymethyl cellulose, hydroxyethyl cellulose, polyethylene glycol with a molecular weight of 300—6,000, polyvinyl alcohol with a molecular weight of 20,000—100,000, and polyvinylpyrrolidone with a molecular weight of 20,000—100,000. 45
10. A process for producing a stable granulocyte colony stimulating factor containing pharmaceutical preparation that contains, in addition to the granulocyte colony stimulating factor present as the effective ingredient, at least one substance selected from the group consisting of a pharmaceutically acceptable surfactant, saccharide, protein and high-molecular weight compound. 50
11. A stable granulocyte colony stimulating factor containing pharmaceutical preparation substantially as hereinbefore described, with reference to Example 1, 2 or 3.
12. A process for producing a stable granulocyte colony stimulating factor containing pharmaceutical preparation substantially as hereinbefore described, with reference to Example 1, 2 or 3. 55

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